Figure 1a

MDSEAFQSARDFLDMNFQSLAMKHMDLKQMELDTAAAKVDELTKQLESLWSDSPAPPGPQAGP PSRPPRYSSSSIPEFFGSRGSPRKAATDGADTPFGRSESAPTLHPYSPLSPKGRPSSPRTPLYLQPDAY GSLDRATSPRPRAFDGAGSSLGRAPSPRPGPGPLRQQGPPTPFDFLGRAGSPRGSPLAEGPQAFFPE RGPSPRPPATAYDAPASAFGSSLLGSGGSAFAPPLRAQDDLTLRRRPPKAWNESDLDVAYEKKPSQ TASYERLDVFARPASPSLQLLPWRESSLDGLGGTGKDNLTSATLPRNYKVSPLASDRRSDAGSYRR SLGSAGPSGTLPRSWQPVSRIPMPPSSPQPRGAPRQRPIPLSMIFKLQNAFWEHGASRAMLPGSPLF TRAPPPKLQPQPQPQPQPQPQPQPQPQPQTQPQTTPTAPQHPQQTWPPVNEGPPKPPTELEPEPEI EGLLTPVLEAGDVDEGPVARPLSPTRLQPALPPEAQSVPELEEVARVLAEIPRPLKRRGSMEQAPA VALPPTHKKQYQQIISRLFHRHGGPGPGGPEPELSPITEGSEARAGPPAPAPPAPPPPPAPSQSSPPEQ PQSMEMRSVLRKAGSPRKARRARLNPLVLLLDAALTGELEVVQQAVKEMNDPSQPNEEGITALH NAICGANYSIVDFLITAGANVNSPDSHGWTPLHCAASCNDTVICMALVQHGAAIFATTLSDGATAF EKCDPYREGYADCATYLADVEQSMGLMNSGAVYALWDYSAEFGDELSFREGESVTVLRRDGPEE TDWWWAALHGQEGYVPRNYFGLFPRVKPQRSKV*

Figure 1b

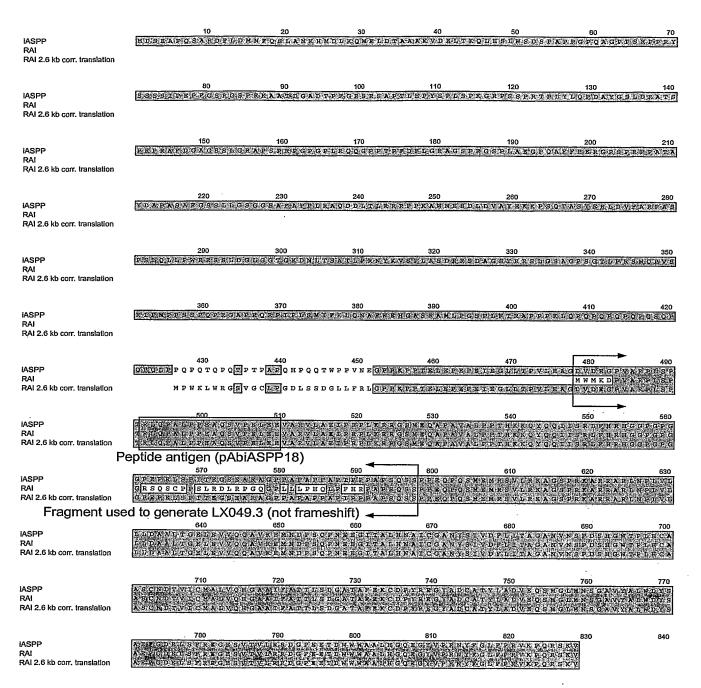
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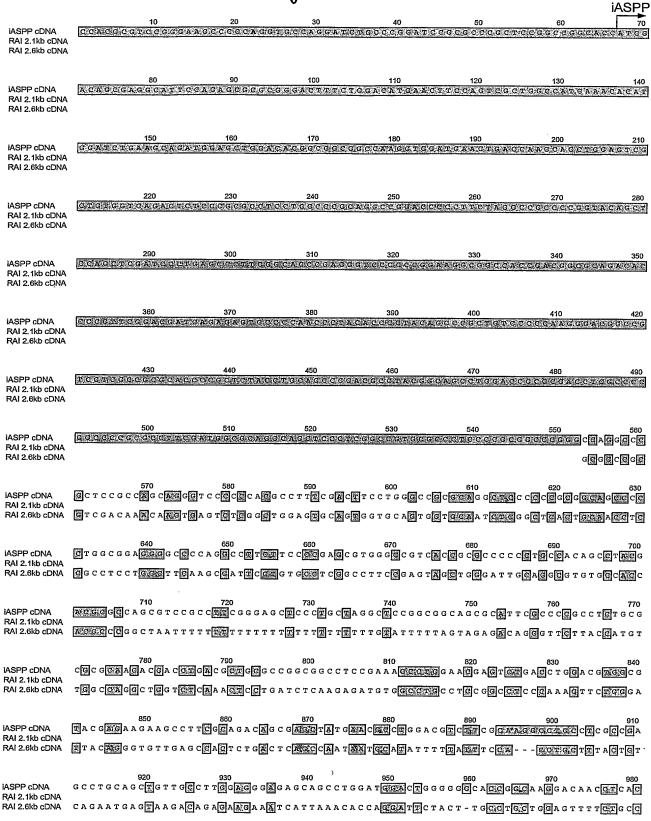
Figure 2a

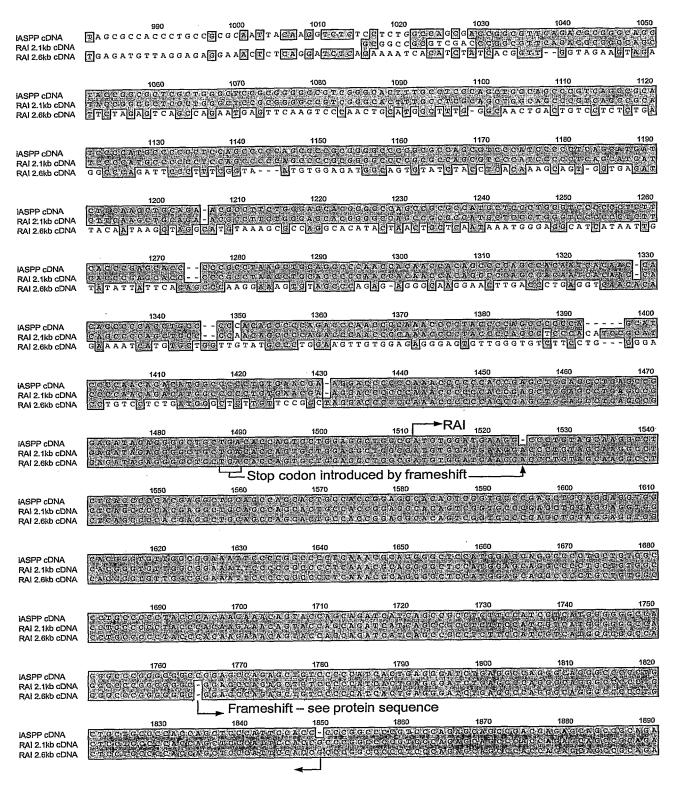
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Figure 2b

GGGCACTTTGCCTCGCAGCTGGCAGCCCGTCAGCCGCATCCCATGCCCCCCTCCAGCCCCAGCCCC GCGGGGCCCGCGCCAGCGTCCCATCCCCCTCAGCATGATCTTCAAGCTGCAGAACGCCTTCTGGGA GCACGGGGCCAGCCGCG CCATGCTCCCTGGGTCCCCCTCTTCACCCGAGCACCCCCGCCTAAGCTG CAGCCCCAACCACACCACAGCCCAGCCACAATCACAACCACAGCCCCAGCTGCCCCAACAGCCC AGACCCAACCCCAAACCCCTACCCCAGCCTCCCACATCCGCATCCCCAACAGACATGGCCCCTGTG AACGAAGGACCCCCAAACCCCCACCGAGCTGGAGCCTGAGCCGGAGATAGAGGGGCTGCTGACA CCAGTGCTGGAGGCTGGCGATGTGGATGAAGGACCCTGTAGCAAGGCCTCTCAGCCCCACGAGGCTG CAGCCAGCACTGCCACCGGAGGCACAGTCGGTGCCCGAGCTGGAGGAGGTGGCACGGGTGTTGGCG GAAATTCCCCGGCCCTCAAACGCAGGGGCTCCATGGAGCAGGCCCTGCTGTGGCCCTGCCCCCTA CCCACAGAAAACAGTACCAGCAGATCATCAGCCGCCTCTTCCATCGTCATGGGGGGCCAGGGCCCGG GGGGCGGAGCCAGAGCTGTCCCCCATCACTGAGGGATCTGAGGCCAGGGCAGGGCCCCTGCTCCTG CCCCAC CAGCTCCCATTCCACCGCCCGGCCCGTCCCAGAGCAGCCCACCAGAGCAGCCGCAGAGC CTCTGGTGCTCCTCGGACGCGGCGCTGACCGGGGAGCTGGAGGTGCAGCAGCCGGTGAAGG AGATGAACGACCCGAGCCAACGAGGAGGGCATCACTGCCTTGCACAACGCCATCTGCGGCG ${\tt CCAACTACTCTATCGTGGATTTCCTCATCACCGCGGGTGCCAATGTCAACTCCCCGACAGCCACGGCCCCGGGTGCCAATGTCAACTCCCCCGACAGCCACGGCCACGGCCCCGGGTGCCAATGTCAACTCCCCCGACAGCCACGCCACGGCCACGGCCACGGCCACGGCCACGGCCACGGCCACGGCCACGGCCACGGCCACGGCCACGGCCACGGCCACGGCCACGCCACGGCCACGCCACGGCCACGCACACGCCACGCCACGCCACGCCACGCCACGCCACGCCACGCCACGCCACGCCACGCCACGCCACACGCCACGCCACGCCACGCCACGCACGCCACGCCACGCCACGCCACGCACGCCACACGCCACGCACACGCCACGCCACACGCCACACGCCACGCCACACACACACACACACACACACACACACACACAC$ TGGACACCCTTGCACTGCGCGCGTCGTGCAACGACACAGTCATCTGCATGGCGCTGGTGCAGCACG GCGCTG CAATCTTCGC CACCACGCTC AGCGACGGCG CCACCGCCTTCGAGAAGTGCGACCCTTACC GCGAGGGTTATGCTGACTGCGCCACCTACCTGGCAGACGTCGAGCAGAGTATGGGGCTGATGAACA GCGGGCAGTGTACGCTCTCTGGGACTACAGCGCCGAGTTCGGGGACGAGCTGTCCTTCCGCGAGGG CGGCCAGGAGGCTACGTGCCGCGGAACTACTTCGGGCTGTTCCCCAGGGTGAAGCCTCAAAGGAGT AAAGTCTAGCAGGATAGAAGGAGGTTTCTGAGGCTGACAGAAACAAGCATTCCTGCCTTCCCTCCAG ACCTCTC CCTCTGTTTTTTGCTGCCTT TATCTGCACC CCTCACCCTG CTGGTGGTGG TCCTTGCCAC CGGTTCTCTGTTCTCCTGGAAGTCCAGGGAAGAAGGAGGGCCCCAGCCTTAAATTTAGTAATCTGCCTTAGCCTTGGGAGGTCTGGGAAGGGCTGGAAATCACTGGGGACAGGAAACCACTTCCTTTTGCCAAA TCAGAT CCCGTCCAAA GTGCCTCCCA TGCCTACCAC CATCATCACA TCCCCCAGCAAGCCAGCCAC TCTCTCCCAGCAGTCTTGGGGTCTGGGTGGGAAACATTGGTCTCTACCAGGATCCCTGCCCCACCTCT CCCCA ATTAAGTGCC TTCACACAGC ACTGGTTTAATGTTTATAAA CAAAATAGAG AAACTGGTTT AATGTTTATA AACAAAATAG AGAAACTTTCGCTTATAAAT AAAAGTAGTT TGCACAGAAA TGAAAAAAA AAAAAAAAAA AAAAAA







iASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	GCATGGAG GCATGGAG	1900 ATCCGCTC ATGCGCTC	1910 IGTGCTGCGG IGTGCTGCGG IGTGCTGCGG	1920 AAGGCGGCT AAGGGGGCT AAGGCGGGCT	1930 C C C C G C G C A A C C C C G C G C A A C C C C G C G C A A	1940 6 G C G C G C G G 6 G C C G G C G G 6 G C G G G C G G	1950 G C G G G C C T C A / G C G C G C C T C A /	1960 A C C C A C C C V C C C
iASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	TOTGGTGC	TCCTCCTG	ACGCGGGG	TGAC'CGGGGA"	GOTGGAGGTG	GTGCTGCATC	2020 C.G.O.T.G.A.A.G.G.A.G. C.G.O.T.G.A.A.G.G.A.G. C.G.G.T.G.A.A.G.G.A.G	37.00
IASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	AIA C G A C G C Ā A C G A C G C	GAGCCAGC	LUAACGAGGA	GGGCATCACT	GCCTTGCCACCA	ACCOUNTOFO	2090 C G G C C A A C T C G G C G C C A A C T C G G C G C C A A C T	1500000
ìASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	CTATCGTG CTATCGTG	GATATTCCTC	ATCACCCCC	SGTGCGAATG	ncaacmooo	CSORCOONO	2160 5 G C T G G A C A C C 5 G C T G G A C A C C 5 G C T G G A C A C C	2 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 -
ìASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	GCACTGCG GCACTGCG	$\mathbf{C} \cdot \mathbf{G} \cdot \mathbf{G}$	GCAACGACA	JAGECATOTO	7 A T'C'C C C C T T C (THE CAMPAGE	2230 SCGCCIGCAATC SCGCTGCAATC	
IASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	G C C A C C A C	250 C C C A G C C A G C T C A G C G A G C T C A G C G A	2260 G G G G G G A C G C G G G G G A C G	2270 G C C T T C G A G C G C C T T C G A G C G C C D T C G A G C	2280 (A G T G C G A C C (A G T G C G A C C (A G T G C G A C C C	2290 ETTAGGGCGCGAG ETTAGGGGGGAG	2300 SIG GITTATECTE SIG STTATECTE	2310 A C T A C T A C T
iASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	G C G C C A C C	320 FACCT GGGA FACCT GGGA	2330 G'A' C'G' T' C'G' A' G' C G'A' C'G T' C' G' A' G' C	2340 LAGAGTATGG LAGAGTATGGG	2350 GCTGATGAAG GCTGATGAAG	2360 A G G G G G C A G A G C G G G G D A G	2370 FT GTA CG CT CT FGTA CG CT CT	2380 C T G C T G C T G
IASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	G G A C T A C A G G A C T A C A	BUBLE CUBAGE	$\mathbf{L}, \mathbf{C}, \mathbf{G}, \mathbf{G}, \mathbf{G}, \mathbf{G}, \mathbf{A}, \mathbf{C}, \mathbf{G}, \mathbf{A}$	COUNTRY OF THE COURT TO THE	CGGGAGGGG	A G:T: C G G:T C A C	2440 C.G.T.O.C.T.G.C.G.G. L.C.T.G.C.T.G.C.G.G.	
IASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	GACGGGCC	60 JGAGGAGACA GAGGAGAC	2470 C.O.A.C.T.G.G.T.G.G. C.G.A.C.T.G.G.T.G.G.	2480 TIG G G G G G G G G TIG G G G G G G G G	2490 T.G.O.A.C.G.G.C.A. T.G.O.A.C.G.G.C.C.A. L.G.O.A.C.G.G.C.C.A	2500 '9 9 A G G G C T A C G G A G G G C T A C	2510 GT G C C G C G G A G T G G C G G A G T G G C G G A	2520 A C T A C T A C T
iASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	The Late Control of the	TGTTCCCC TGTTCCCC	AGGGLGAAGC	CTCAAAGGAG	TA A A COT COT A Y	CACCAMPACA	2580 G G A G G T T T C T T G G A G G T T T C T C	200
IASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	26 G G T G A C A G N G G T G A C A G A G C T G A C A G A	AACAAGCA AACAAGCA	2610 TJCCTGCCTT TTCCTGCCTT	2620 CCCTCCAGAC CCCTCCAGAC	stop 2630 CTICT CCCTOT CTICT CCCTOT CTICT CCCTOT	GTTTTTTCCT	2650 GCCTTTATCT GCCTTTATCT	2660 G.C.A. G.C.A. G.C.A.
iASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	26 C C C T C A G C C C T C A G C C C T C A G C	Слестест Ттестест	2680 G T G G T C C T T G T G G T C C T T	2690 GCCACICGTT GCCACCGTT GCCACCGGTT	2700 CTGTGTTCTC CTCTGTTGTC CTCTGTTCTG	2710 CIGGAAGICC CIGGAAGICC	2720 A G G G A A G A A G A G G G A A G A A G G A G G G A A G A A G G	2730 3 A G 3 A G 3 A G
IASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	27/ GGCCCCAGG GGCCCCAGG	CTTAAATT	2750 DAGIAATCIG AGIAATCIG	2760 C.C.T. T.A.G.C.G.T.T. G.C.T. T.A.G.C.G.T.T. G.C.T. T.A.G.C.C.T.T.	2770 G G G A G G T G T G G G G A G G T C T G G G G A G G T C T G	2780 G G A A G G G C T G G G A A G G G C T G	2790 GAAAT CACT G GAAAT CACT G	2800 G G G G
IASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	281 A Congression A Congression A Congression A Congression	ashed in a comb	2820 TTTGCCAAAT TTTGCCAAAT	2830 CAGATCCCGGT CAGATCCCGGT	2840 C C A A A G T G C C C C A A A G T G C C	2850 T.C.C.C.A.T.G.C.C.T. T.C.C.C.A.T.G.C.C.T.	2860 ACCAOCATCAT ACCACCATCAT	2870 -C.A -C.A -C.A

		2880	2890	2900	2910	2920	2930	2940
IASPP cDNA RAI 2.1kb cDNA RAI 2.6kb cDNA	CATCCCC	CAGICAAGCCA CAGICAAGCCA CAGCAAGCCA	GCCACCTGCC	CAGCCGGGCC	TGGGATGGG	CACCAGACGA	CTGGATATT	CTG
IVAI 2,000 CDIVA	<u> (0,6)1,50,000</u>		B.C.C.A.C.C.II.G.C.C	CA GEGERAL	THE GRANT GEGGE	CCACCCACCA	CDGGATALIT	Soft e
IASPP cDNA	CCACTCA	2950 CTGCTGACAC	2960	2970	2980	2990	3000	3010
RAI 2.1kb cDNA RAI 2.6kb cDNA	GGAGTCA	CTGCTGACAC CTGCTGACAC	CATCICICCC	AGCAGTCTTG	GGGTCTGGGT	GGGAAACATI	GGTCTCTACC	AGG
		3020	3030	3040	3050	3060	3070	3080
iASPP cDNA RAI 2.1kb cDNA	ATCCCTG	CCCACCICT	CCCCAATTAA	GTGCCTTCAC	ACAGCACTIGG	TTTAATGTTT	ATARACAAA	TAG
RAI 2.6kb cDNA	ATECCTS	CCCCACCT	CCCCAATTAA	GTGCCTTCAC	ACAGCACTC	ATTAAT GREE	ATĀĀĀCĀĀĀĀ	TAG
		3090	3100	3110	3120	3130	3140	3150
IASPP cDNA	AGAAACIT				T.T.C.C.TET.A	TAAATAAAG	TAGDTTGCAC	AGA
RAI 2.1kb cDNA RAI 2.6kb cDNA	A G A A A C T	GGTTTAATGT	TATA TA ASA CA A	AATAGAGAAA		TAAATAAAG TAAATAAAAG		
	•							
iASPP cDNA	AIA A A A A	3160 A A A A A A A	3170	3180	3190	3200	3210	3220
RAI 2.1kb cDNA	AATGAAA	AAAAAAAAAA	A A A A A A A A A	A				
RAI 2.6kb cDNA	AAAAAA	AAACACAAC	CTTCGGCCGC	CCCACCAAGC	WOTGT CTCCA	A A A A A A A A A A A A	A, A, A, A, A, A, A, A, A, A	A'A'A
		3230	3240	3250	3260	3270	3280	3290
IASPP cDNA RAI 2.1kb cDNA								
RAI 2.6kb cDNA	A					•		

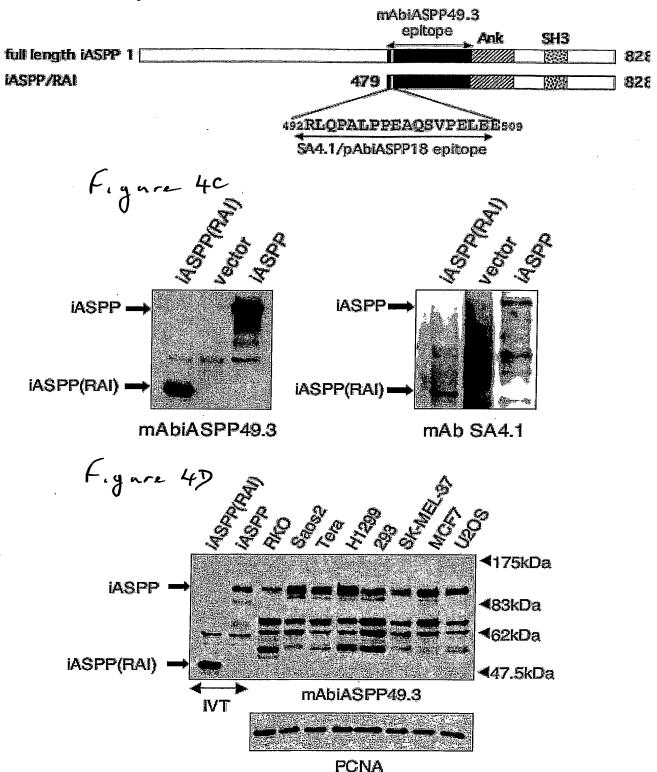
Figure 4a

Expression of iASPP in various cell lines

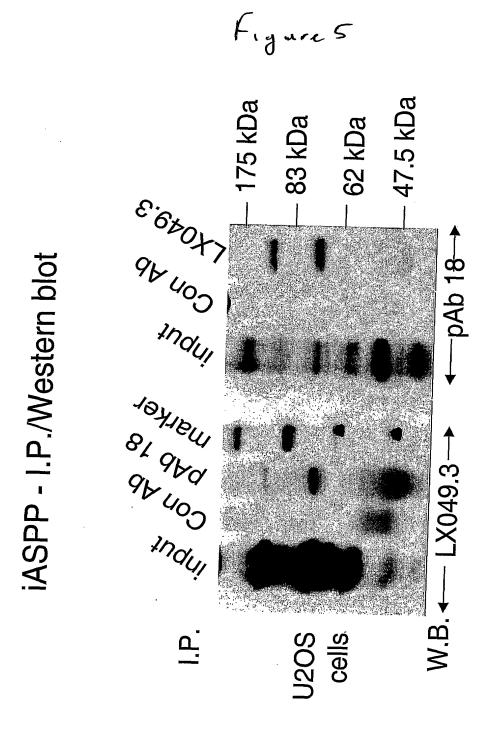
62 kDa 47.5 kDa 83 kDa

Antibody = LX049.3

Figure 4B

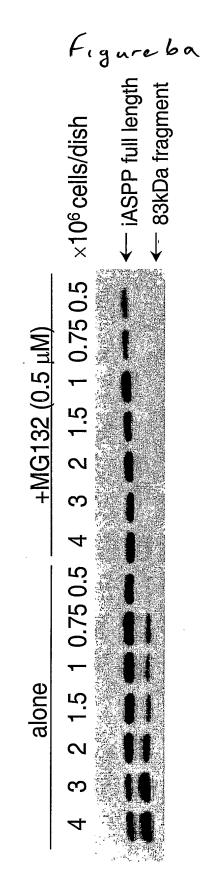


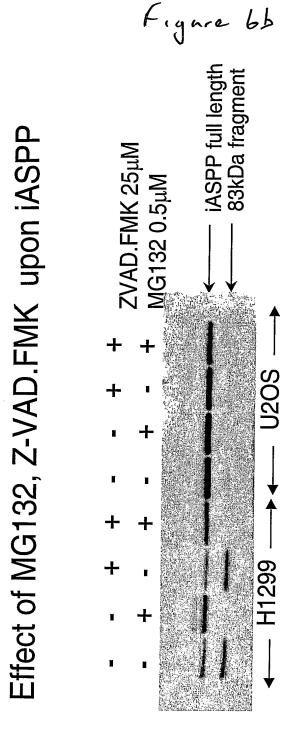
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Effect of cell density and MG132 upon iASPP expression in U2OS cells



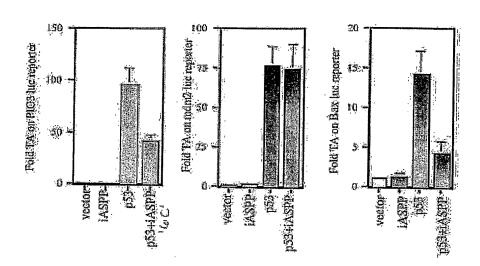


W.B.: α-iASPP (LX049.3)

Interaction of iASPP with p53 and Bcl2 in U2OS cells Input Control Ab (CMT) (CMT) Longer exposure Short exposure Cisplatin 2.5µg/ml iASPP full length 83kDa fragment 75kDa fragment iASPP full length 83kDa fragment 75kDa fragment

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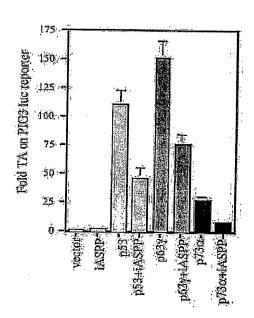


Figure 9

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minigaio Ποχοταρίζη (bateatin)

Marker 800 HISYLTVI

uneids:

Cisplatin Doxorubicin DaneaunU

IAL!YZBB QC

DatestinU nusiqsiQ Doxonpichu Untreated bejaanin()

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